I. A STUDY OF LIVER AND BLOOD CATALASE IN LEUCEMIC AND TUMOR-BEARING ANIMALS

II. EXPERIMENTS WITH L-AMINO ACID OXIDASE

BY

BENJAMIN WEISS

B.S., State University of Iowa, 1944

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1949

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I. CATALASE

A. Liver Catalase

Introduction

The dynamic equilibrium of the normal metabolic process is disturbed when a tissue becomes neoplastic. It has been shown by Greenstein and coworkers (1) that the liver catalase activity was markedly reduced in rats bearing transplants of either hepatic tumor No. 31 or the Jensen sarcoma. Further, it was found that the phenomenon was reversible. The excision of the hepatic tumor transplant permitted a return of the liver catalase activity to the normal level within a relatively short time. When fresh tumor tissue was re-inoculated into animals from which the first tumor had been excised, there occurred again a lowering of the liver catalase. After excision of the second transplant, the enzyme activity returned again to normal (2). The mechanism of this effect is unknown. However, it appears that the inhibitory effect is exerted on the process which controls the synthesis of the enzyme in a tissue rather than a circulating toxic factor which directly inhibits the catalase molecule (3).

In 1948, Miller (4) reported that acute inanition in rats resulted in a loss of liver enzyme activity, including catalase.

The present investigation was undertaken to study further the liver catalase activity of normal and tumor-bearing animals.

Experimental

In this study, C58 mice[1] which show a high incidence of spontaneous lucemia, and mice[1] inoculated with the Hall sarcoma were employed. Histological examination of the liver

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of each animal was made and where there was evidence of infiltration, the method of Chalkley (5) was used to determine the extent.

The apparatus employed for the determination of catalase activity was essentially like that reported by Maisin and Pourbaix (6). The oxygen liberated from the splitting of hydrogen peroxide by the catalase of the tissue extract is measured in a gas burette as a function of time.

Summary

1. *In vitro* experiments indicate that C58 mouse livers infiltrated with malignant lymphocytes possess a diminished catalase activity, becoming further depressed as the extent of infiltration increases. Beyond a certain upper limit of infiltration, no further decrease in liver catalase activity occurs.

2. Mice bearing the Hall sarcoma exhibit a lowered liver catalase activity which diminishes with the progressive growth of the tumor. In all cases, the appearance of the liver was normal.

3. Normal mice subjected to acute inanition for 4 to 5 days, being given water ad libitum, showed no diminution of liver catalase.

4. No marked sex differences regarding catalase activity in normal mice were noted.

B. Blood Catalase

Introduction

It was early recognized that the distinct changes in the character of the blood as the result of malignant growth might serve as a practical diagnostic test. In those cases where the tumor was inaccessible, hidden in some remote portion of the body, the blood might act as an indicator denoting the progressive development of the disease. Deutsch and Frankl

announced a method for detecting cancer by means of blood catalase determinations but, later, withdrew their claim (7), reporting that only slight differences were present in the blood catalase activity of normal and leucemic individuals. In 1942, Greenstein and Andervont (8) investigated the blood catalase activity of Buffalo rats bearing the transplanted Jensen sarcoma and found little difference between normal and tumor-bearing animals. The purpose of this study was to extend the previous observations and to attempt a slightly different approach to the problem of blood catalase activity.

Experimental

The procedure for the determination of blood catalase was similiar to that employed for liver. When no significant differences were observed between the blood catalase activities of normal and leucemic[2] patients, it was decided to test the catalase activity afte rthe application of a physical stress. It was reasoned, that if a difference existed in the manner in which their catalase molecules were assembled, then subjection to heat might result in a rapid inactivation of one while leaving the other untouched or only slightly affected.

Summary

1. The observation that little or no difference exists between the blood catalase activities of normal and leucemic individuals was further confirmed.

2. The catalase molecules of normal and leucemic individuals are equally sensitive to stress, and subjection to heat causes in each a similar degree of inactivation.

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II. L-AMINO ACID OXIDASE

Introduction

The first experimental evidence that the natural amino acids are oxidized to their corresponding keto acids and ammonia was made by Neubauer and his colleagues (1) from perfusion studies. Ever since, an extensive growth of information regarding the individual amino acids has accumulated, but practically nothing was known concerning the nature and number of enzymes which might be involved in their metabolism. In 1944, Blanchard, et. al. (2) showed that the oxidative deamination of approximately half of the naturally occurring amino acids is catalyzed by a single enzyme, L-amino acid oxidase. This enzyme was shown to be a flavoprotein whose prosthetic group was riboflavin phosphate. When oxygen was excluded from a system containing L-amino acid oxidase and substrate, the yellow color, characteristic of the oxidized state of the enzyme, was discharged to the colorless form. If oxygen was afterwards admitted, the original yellow color was restored with the simultaneous formation of hydrogen peroxide.

It was the intention of this investigation to explore the

possibility of obtaining a convenient method for the assay of L-amino acid oxidase, and since no values for this enzyme have been reported in the literature, to make a comparative study of the liver L-amino acid oxidase activity of certain normal and tumor-bearing animals.

Experimental

The procedure, with slight modification, of Blanchard and coworkers, employing the Warburg apparatus, was used to follow L-amino acid oxidase activity. When no oxygen uptake was observed with liver or kidney homogenates, another method for following the course of its activity was developed. This involved incubation of the homogenate with substrate (L-leucine), cleavage of the enzyme and precipitation of the protein with trichloroacetic acid, and finally, determination of the quantity of reduced coenzyme in an Evelyn colorimeter. All operations were carried out under anaerobic conditions.

Summary

1. The activity of L-amino acid oxidase in rat kidney and liver homogenates and extracts was too low to be measured in a Warburg apparatus.

2. A new method was deveolped for measuring L-amino acid oxidase activity in which the concentration of enzyme was determined under anaerobic conditions.

3. Employing the anaerobic method, the activity of L-amino acid oxidase was too low to be determined in rat kidney homogenate. The method proved satisfactory for the determination of pure riboflavin.

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VITA

The author was born on November 16, 1922 in Newark, New Jersey. There he attended the public schools and was graduated from Central High School in February, 1941. In the same year, he entered the State University of Iowa where he received his Bachelor of Science degree in Chemistry in 1944. Following a period of service in the United States Army and employment, he entered the Graduate College of the University of Illinois in February, 1946. From September of that year to June, 1949, he held a Teaching Assistantship.